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EFFECT OF MORPHINE AND NALOXONE ON LH RESPONSE AND SEXUAL BEHAVIOR OF RAMS (*OVIS ARIES*)

J.A. Fitzgerald[1,2,3] and A. Perkins[4]

USDA-ARS, Dubois, Idaho 83423

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ABSTRACT

Effects of the opiate agonist, morphine, and antagonist, naloxone, on LH release, courtship behavior and ejaculation frequency of mature, sexually active or sexually inactive rams were investigated. Plasma LH concentrations were monitored from blood samples collected every 15 min for 10 hr (0800 to 1800 hr) from eight rams that were isolated from or in contact with estrous females. Plasma LH concentration was higher ($P<.05$) in sexually active rams exposed to receptive females compared with hormone concentration of rams isolated from ewes. Intravenous infusion of morphine sulphate (1 mg/kg) into rams 4 and 6 hr after exposure to ewes reduced ($P<.05$) plasma LH concentration as compared to rams given saline. Morphine did not affect ($P>.05$) courtship behavior (investigatory sniff, mount attempt, foreleg kick, flehmen, vocalization) but diminished ($P<.05$) number of ejaculations. In another trial, LH concentrations were higher ($P<.05$) in seven sexually active rams given naloxone *iv* or when given to three rams through an intracerebroventricular cannula (*icv*) as compared to LH response of sexually inactive rams. LH did not differ ($P>.05$) in seven sexually inactive rams before or after administration of naloxone. Investigatory sniffs by sexually active rams were increased ($P<.03$) after treatment with the opiate antagonist. Four of the seven sexually active rams had more ejaculations after naloxone compared with the pretreatment period, but mean ejaculation frequency after treatment did not differ ($P=.31$). Naloxone did not stimulate courtship behavior of sexually inactive males. These data suggest that the effect of opiates on sexual behavior and LH secretion depends upon the inherent level of sexual activity among rams.

INTRODUCTION

Opioid peptides have been implicated in the control of LH release in rams (1,2). In general, opiate agonists inhibit, while antagonists enhance, plasma LH concentration. Evidence demonstrating a role for endogenous opiate activity influencing sexual behavior has been collected for several species (3) yet the relationship between opiates and sexual behavior in rams has not been thoroughly investigated. Rams, classified by serving capacity tests into groups having high and low sexual activity, have markedly different plasma LH concentration when kept in the presence of estrous ewes (4). There is a positive association of level of sexual activity to LH release. This parallel relationship among rams of low sexual activity and reduced LH or high sexual activity with increased LH response suggested to us that endogenous opioid peptides may play an important role in mediating these interrelationships. We hypothesized that low sexual activity was associated with increased endogenous opioid activity. The objective of this study was to compare the effects of an opiate agonist, morphine, and an opiate antagonist, naloxone, on sexual behavior and plasma LH concentrations in rams.

MATERIALS AND METHODS

Rams. The Location Animal Care Committee of the U.S. Sheep Experiment Station approved the following studies. Rambouillet and Targhee rams, 3 to 6 yr of age, were kept under ambient photoperiod (49°18' N Latitude) and temperature. Serving capacity tests were performed as previously described (5) to characterize sexual activity. Rams with average libido achieved from 2 to 4 ejaculations in 30 min for 6 separate tests con-

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ducted during the autumn breeding season. Rams with low libido averaged 1 ejaculation, or failed to service estrous ewes (sexually inactive males).

Ewes. Ovariectomized ewes were induced into estrus by treatment for 7 d with vaginal pessaries containing progestin (MAP,60 mg, Tuco Products, Orangeville, Ontario, Canada) followed by two injections of estradiol (50 μg, corn oil) spaced 24 hr apart beginning when pessaries were removed. Estrous behavior occurred within 48 hr after the first estradiol injection.

Blood collection. Each ram was fitted with an indwelling jugular vein cannula (Angiocath, Deseret Medical, Salt Lake City, UT) 24 hr before the collection of blood samples. Blood samples (4 ml) were taken every 15 min for 10 hr (0800 to 1800 hr). Samples were collected from each ram both after being isolated from ewes for one week and in the presence of estrous females. The blood collection periods occurred within ten d.

Courtship behaviors. Rams were placed in individual pens (10.4 m^2) and exposed to three estrous ewes from 0800 to 1800 hr. Groups of ewes were interchanged every 2 hr to maintain the sexual interest of rams. Four observers recorded courtship behaviors and number of ejaculations from each of 2 adjacent test pens. Courtship behavior was recorded continuously and included incidence of investigatory sniffs of the anal-genital region, vocalizations, flehmen, foreleg kicks, mount attempts and mounts.

Drug treatments. To determine if ejaculation frequency or LH release were affected by opiates in sexually active males, morphine sulphate (Eli Lilly, Indianapolis, IN) or physiological saline was given to each of four sexually active rams 4 hr and 6 hr after ewes had been introduced into their pens. Morphine (1.0 mg/kg body weight) in 10 ml of physiological saline was infused through a jugular cannula over a 2 min interval. Frequent blood samples were taken and courtship behaviors were monitored as previously described. In another trial, naloxone hydrochloride (Sigma Chemical, St. Louis, MO) was given to seven sexually active rams or seven sexually inactive rams at a dose of 1.5 mg/kg body weight in 3 to 5 ml of saline. In addition naloxone (100 μg) was given 4 and 6 hr after ewe exposure to another group of sexually active rams (n= 3) using a cannula permanently implanted into the lateral ventricle of the brain (*icv*). Injections were given as a single bolus using a gas tight Hamilton syringe at a volume of 50 uL. The injection needle was calibrated to extend 1 mm beyond a guide cannula.

Surgical procedures. A permanently implanted cannula was placed into the lateral ventricle of the brain by a stereotaxic surgical procedure. Rams were given a pre-anesthetic of 10 mg Rompun (Miles Labs, Shawnee, KA) 15 min before administration of 800 mg Ketamine (Aveco Inc., Fort Dodge, IA), *iv.* A tracheal tube was inserted and general anesthesia maintained with 4 to 5% methoxyflurane (Pittman-Moore, N.Y). The head was positioned into a Kopf stereotaxic apparatus designed for sheep. The skull was exposed by a mid-line incision of approximately 10 cm. The anatomical site of Bregma was located. A micromanipulator holding the cannula was positioned and coordinate readings of Bregma taken. After calculating the target from the Bregma and ear bar zero coordinates, a hole was drilled to the surface of the brain using a Dremel (Racine, WI) drill and 4 mm bit. A stainless steel cannula (38 mm length, 14 gauge needle stock) equipped with a stylet (16 gauge needle stock) was lowered dorsal-ventrally to the target. After placement of the cannula, the stylet was removed. Flow of CSF was checked by inserting an inner guide tube (14 gauge Teflon) filled with sterile saline and attached to a 3 ml syringe. The syringe was removed and once flow was verified, the stylet was replaced and the cannula closed with a screw cap top. The cannula was fixed permanently to the skull with dental acrylic. Four screws tapped into the skull and connected by stainless steel wire were used to give the acrylic more rigid support. Rams were given 100 mg Banamine (Scherring, N.Y.) and a 2.5 mg oral bolus of Albon (Hoffman-La Roche, Nutley, NJ). Body temperature measurements were recorded daily for 7 to 10 d

and antibiotic administered as needed. Phenylbutazone (Vedco, St. Joseph, MO) boluses (1 g) were administered for the initial week postoperative. Rams were used for the studies within 6 wk to 8 wk after surgery. Libido was tested before and after surgery and was not affected by the surgical procedure. At the completion of the studies, rams were killed by lethal injection of pentobarbital. The position of the cannulae was verified by gross dissection.

Hormone assays. LH concentration was measured in each plasma sample in duplicate by RIA (6) using LH antiserum (NIADDK) and purified ovine LH (LER-1056-C2). Inter-assay CV from 4 assays was 12.2 ± 1.3%. Intra-assay CV of a plasma sample containing a known concentration of LH was 9.7 ± .3%.

Statistical analysis. Data were analyzed using General Linear Models Procedures of SAS (7). LH concentration was analyzed by repeated measures ANOVA (8). The analysis of LH included the effect of time (sample collection), treatment (drug vs. control) and interaction. The ANOVA of courtship behaviors included the effects of treatment (morphine, naloxone). Mean differences were compared using Tukey's test.

RESULTS

Effect of morphine on LH and sexual behavior. Mean LH concentration was approximately 2-fold greater (P<.05) in rams exposed to estrous females compared to rams isolated from ewes (Figure 1 and 2). Mean LH from individual rams ranged from .4 to .7 ng/ml during a 4 hr period when samples were collected in the absence of females. Conversely, LH increased in 5 of 8 rams during the first 4 hr of courtship to levels greater than 1 to 2 ng/ml and were above levels of isolated rams over the next 4 hr interval. Administration of morphine 4 and 6 hr after ewe exposure reduced (P<.05) the stimulatory effect of the female on LH. Mean LH concentrations after morphine were similar to levels of rams isolated from ewes. Morphine did not change courtship behaviors of rams (Table 1) but reduced ejaculation frequency (P<.05).

Effect of naloxone on LH and sexual behavior. Naloxone given *iv* or *icv* to sexually active rams stimulated (P<.05) LH release in the presence of estrous (Figure 3 and 4).

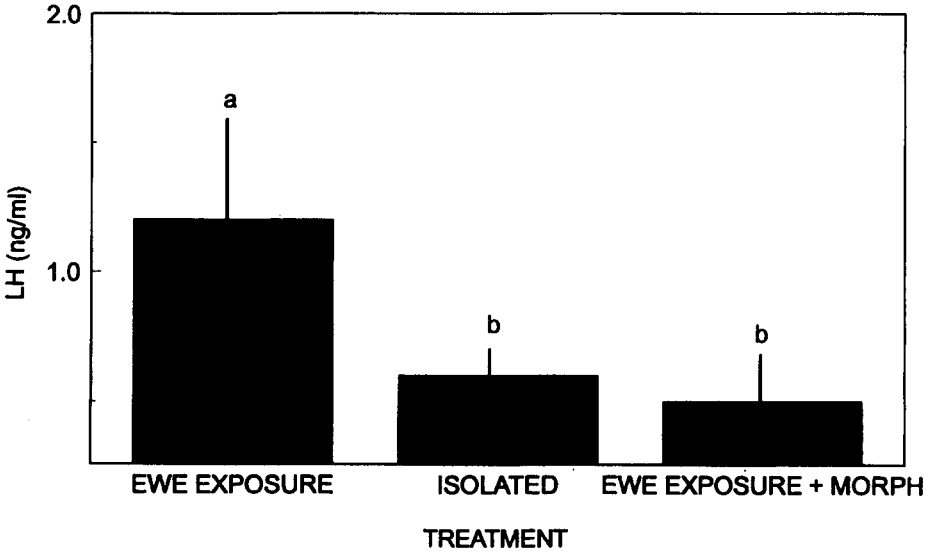

Figure 1. Effect of estrous ewes and morphine on LH release of sexually active rams. Means based on eight sexually active males exposed to or isolated from ewes . Four of the rams were given two injections of morphine sulphate four and six hr after ewe exposure. Morphine reduced (P<.05) the ewe effect on LH release.

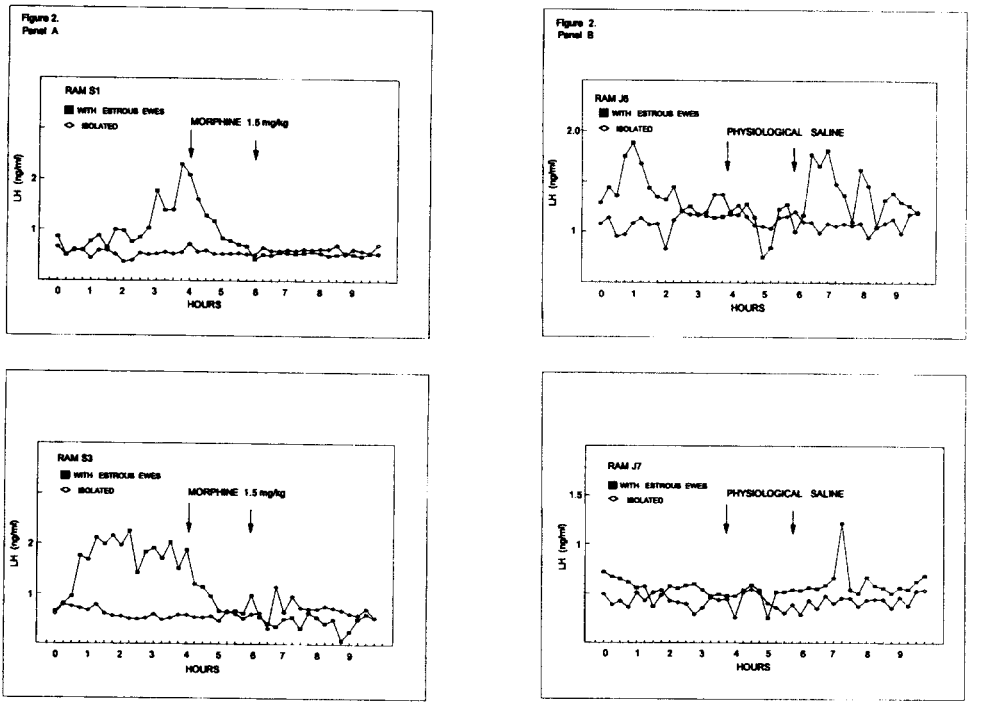

Figure 2. Representative profiles of LH release from sexually active rams treated with morphine sulphate (Panel A) or physiologic saline (Panel B) four and six hr after ewe exposure. LH concentration of each ram during isolation from estrous ewes is given for reference. Ewes were introduced after the initial blood sample was collected and a new group introduced to each ram every two hr.

TABLE 1. MEAN (±SEM) NUMBER OF COURTSHIP BEHAVIORS AND EJACULATIONS OF RAMS GIVEN MORPHINE (N=4) OR PHYSIOLOGIC SALINE (N=4).

	Interval (hr) from treatment			
	Saline Treated		Morphine Treated	
Behavior	Pre (−4hr)	Post (+4hr)	Pre (−4hr)	Post (+4hr)
Anogenital sniff	64 ± 11	42 ± 6	60 ± 16	65 ± 16
Foreleg kick	100 ± 10	87 ± 17	70 ± 14	55 ± 12
Mount attempt	7 ± 2	5 ± 1	4 ± 1	2 ± 1
Mount	23 ± 6	15 ± 2	28 ± 1	12 ± 6
Vocalization	89 ± 12	141 ± 10	70 ± 9	118 ± 6
Flehmen	7 ± 1	4 ± 1	3 ± 1	4 ± 1
Ejaculation	6.3^{a} ± 1.4	2.4^{b} ± .3	4.5^{a} ± .3	1.1^{c} ± .2

[a,b,c]Row means with different superscript ($P < .05$).

Both pulse frequency and baseline LH concentration increased after treatment with the opiate antagonist when compared to the 4 hr interval preceding administration of the drug. The stimulatory effect of naloxone (*iv*) on LH release was related to level of sexual activity (Figure 5 Panel A,B). There was a clear stimulatory effect of naloxone on LH response, with sexually active rams having a greater overall mean LH concentration after treatment ($P<.05$) compared with sexually inactive rams. Ram number 15, classified as inactive on the day of exposure to ewes and collection of blood samples, had shown courtship activity in previous serving capacity tests. It is interesting to note that this individual had the greatest increase in LH release after naloxone compared to other sexually inactive rams.

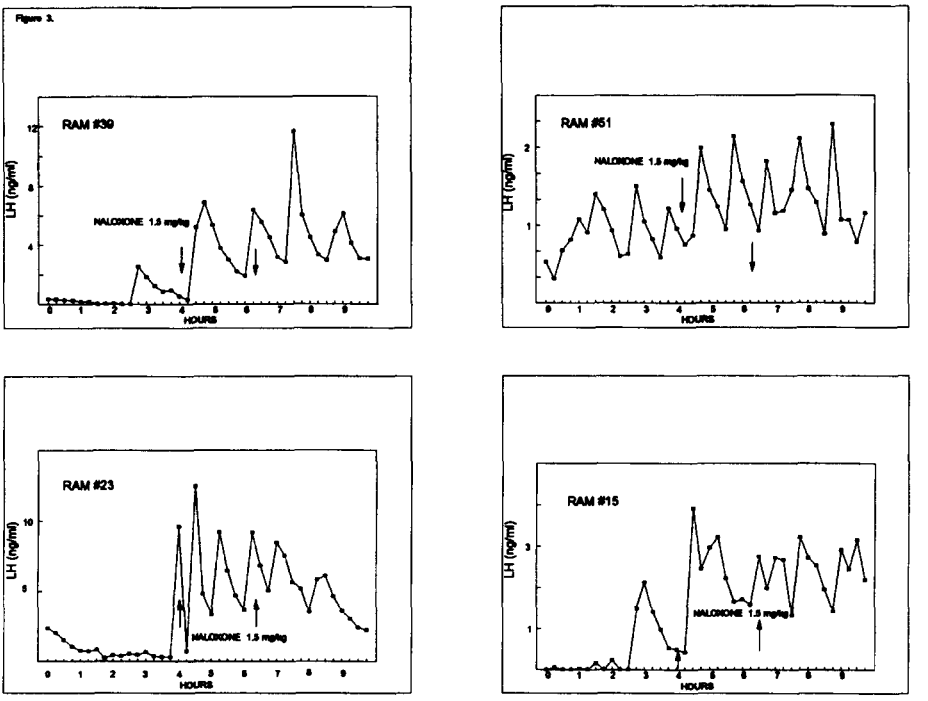

Figure 3. Representative profiles of LH release from rams given naloxone, *iv* (arrows). Rams were in pens with three estrous ewes throughout the sampling period.

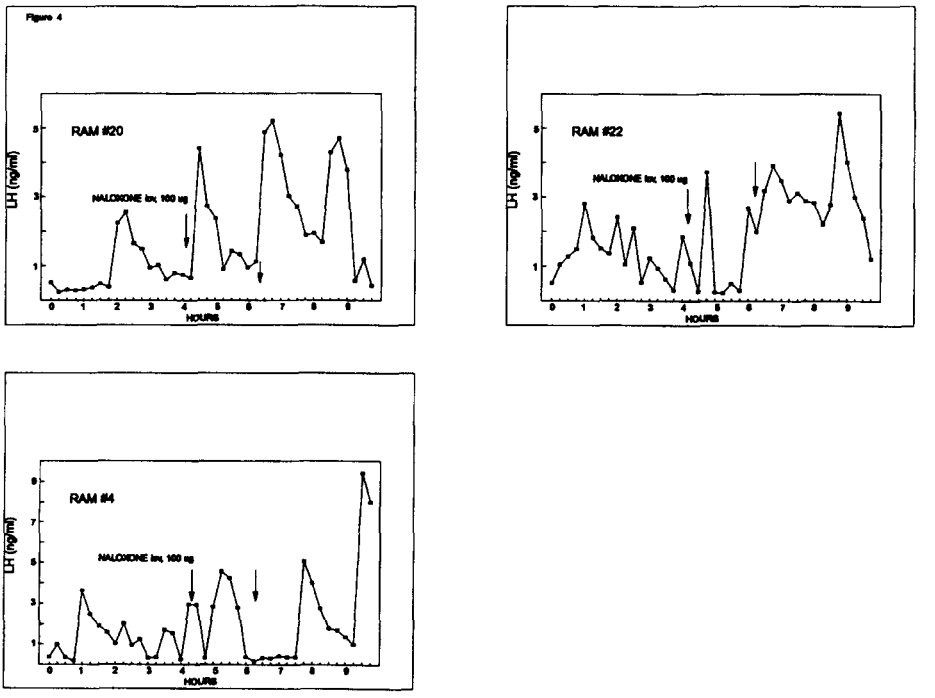

Figure 4. LH release of individual rams given naloxone, *icv* (arrows). Naloxone was given to each ram four and six hr after exposure to females.

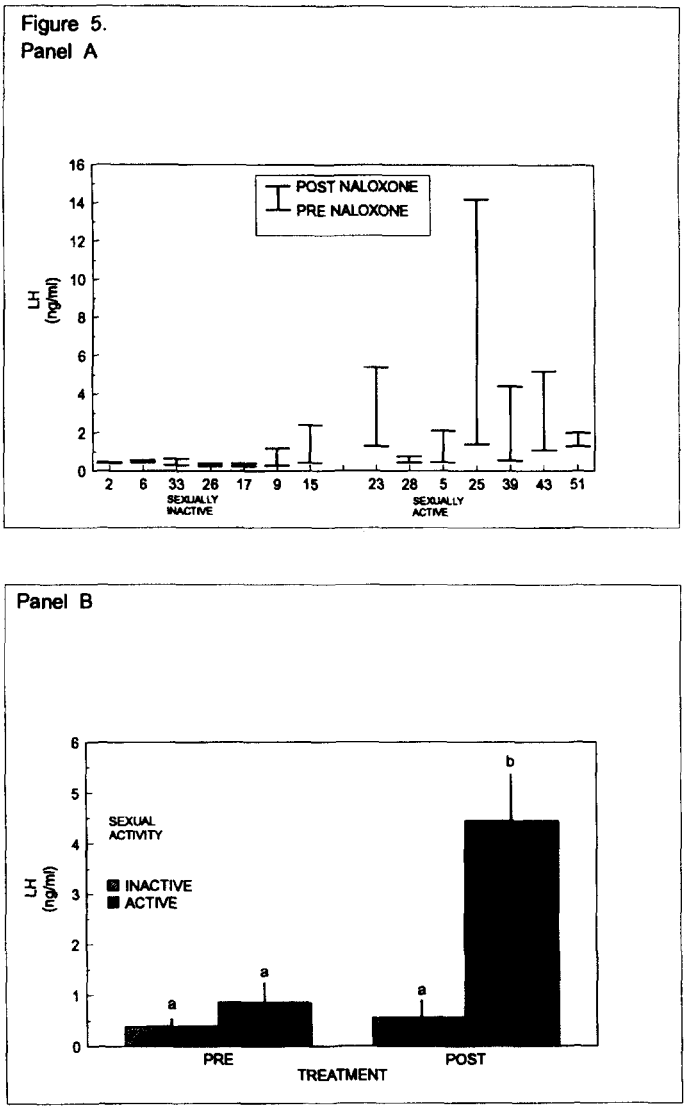

Figure 5. LH release of sexually active and inactive rams (Panel A) given naloxone *iv*. The horizontal bars indicate mean LH concentration four hr before naloxone and after treatment with opiate antagonist. The vertical line shows differences between means (response to treatment) for each ram. Mean LH (Panel B) was greater ($P<.05$) in sexually active compared to sexually inactive rams after treatment with naloxone.

TABLE 2. MEAN (±) NUMBER OF COURTSHIP BEHAVIORS AND EJACULATIONS OF SEXUALLY ACTIVE RAMS (N=7) BEFORE AND AFTER TREATMENT WITH NALOXONE.

Behavior	Before	After
Anogenital sniff	$72^a \pm 6$	$100^b \pm 8$
Foreleg kick	71 ± 23	92 ± 19
Mount attempt	12 ± 8	16 ± 8
Mount	25 ± 8	19 ± 5
Vocalization	165 ± 59	218 ± 79
Flehmen	9 ± 2	7 ± 2
Ejaculation	8.7 ± 1.0	11.1 ± 2.1

[a,b]Row means with different superscript ($P < .03$).

Number of investigatory sniffs were greater (P<.03) in rams given naloxone (Table 2). Average number of ejaculations did not differ (P=.31) for rams given naloxone *iv*, although 4 of 7 rams had more ejaculations after treatment. One of 3 rams given naloxone *icv* had an improvement in ejaculation frequency from 4 to 14 services. The other two rams however did not differ in ejaculation frequency before (5.0 ± .5) and after (7.0 ± 3.7) treatment. Naloxone did not induce courtship behaviors in any of the seven sexually inactive males. Inactive rams remained passive in the presence of the estrous females throughout the experimental period.

DISCUSSION

The positive influence of the female on LH release has been demonstrated for several species including the ram (3). In the present study plasma LH was similar to our previous report (4). LH concentration increased during courtship and mating. One question of interest is whether the stimulatory effect of the female on LH secretion in rams is mediated by changing pituitary sensitivity to LHRH, or by changing the frequency of LHRH secreted (9). In the present studies morphine clearly abolished the stimulatory effect of the ewe on LH release in sexually active rams. Naloxone, an opiate receptor antagonist, evoked an increase in LH release when these rams were exposed to estrous ewes. Morphine has been shown to inhibit LH release in sexually active rams, wethers and wethers given testosterone (2) but does not reduce LHRH-mediated LH release. This suggests that females stimulate LH secretion of rams through increased release of LHRH from the hypothalamus rather than by changing pituitary sensitivity to LHRH.

Morphine at the dose tested did not affect the courtship behaviors monitored in this study. Rams actively courted ewes in both treated and control groups. The tendency for some mating behaviors and ejaculation to decline over time was not unexpected (10) but was likely attenuated by interchanging ewes among rams. Nonetheless, rams given morphine had fewer ejaculations than untreated rams. These data are consistent with observations in other species (3) showing an inhibitory effect of morphine on ejaculation rate of intact, adult males.

Gessa et al. (11) have reported that naloxone stimulates courtship behaviors and improves ejaculation frequency when given to sexually inactive rats. We did not observe a similar response when the opiate antagonist was administered to the sexually inactive ram. Four rams that were sexually active before treatment with naloxone had more ejaculations after treatment but mean ejaculation frequency was not statistically improved by naloxone. In studies with rodents (12), facilitation of courtship and ejaculation frequency by naloxone may be related to the dose of the drug administered. We chose a level of the drug that had been reported to be effective in stimulating LH secretion of rams during the breeding season (2) but was twenty fold less on a body weight basis than that administered to rats by Myers and Baum (12). Number of investigatory sniffs were greater in rams given naloxone which suggests some effectiveness of the opiate antagonist at the dose given. A higher dose of naloxone or imposing treatments of a longer duration may have been required to show a statistically significant effect on ejaculation frequency. We chose to test the effect of naloxone independent of morphine pre-treatment. Some studies showing a positive effect of the opiate antagonist on ejaculation rate involve reversal of inhibition by pre-treatment with morphine (3). Agmo and Paredes (13) showed that naloxone affected sexual behavior of rats if it was after pre-treatment but was ineffective without pre-treatment.

Our observation that naloxone failed to facilitate ejaculation frequency may also be related to site of action. Evidence of facilitation of sexual behavior by naloxone at the spinal level has been reported for the rat (14). The peripheral effect is hypothesized to

involve an effect on sensory transmission during copulation. If the effect of naloxone on facilitation of ejaculation in the ram is also mediated peripherally and not primarily central through sexual arousal, then this may help explain why naloxone was ineffective when given to sexually inactive rams that were not copulating but did stimulate some rams that were sexually active. Although we did inhibit ejaculation frequency with morphine, the failure to demonstrate a clear facilitation of naloxone on ejaculation in the present study does not support the hypothesis of increased opioid tone as a causative factor in sexual inactivity of rams.

The dependence of the effect of naloxone on LH release with level of sexual activity may have some application for screening rams. Ewe induced LH release has been shown to be greater in rams with higher scores in serving capacity tests (4). The present observation of an association of naloxone responsiveness with level of sexual activity provides additional evidence that the ewe effect is translated through LH release and correlates with serving capacity test results. Refinement of this method could result in a blood test in lieu of multiple serving capacity tests. Use of a blood test might reduce variability (15) associated with choosing breeding rams.

In summary, observations from these studies collectively suggest that the effect of opiates on sexual behavior and LH secretion depends upon the inherent level of sexual activity among rams. The estrous ewe stimulates LH secretion of sexually active males. During the breeding season, sexually inactive rams do not respond to estrous ewes with any appreciable changes in LH secretion (5). Perception of the estrous ewe by rams that differ in sexual activity is translated differently in terms of LH secretion. When sexually active rams were treated with morphine, both ejaculation frequency and LH secretion were reduced. Conversely, LH release of sexually active rams was increased when rams were treated with naloxone. Effect of the opioid peptides on LH secretion is likely mediated through central neuroendocrine pathways. We originally hypothesized that a higher endogenous opioid tone would explain the association of lowered LH release with diminished serving capacity. The increased responsiveness of LH secretion of sexually active rams compared with that of sexually inactive rams lends support to this hypothesis as it relates to gonadotropin release. Yet naloxone did not reverse complete sexual inactivity nor facilitate an increase in ejaculation frequency of sexually active rams in all cases. Taken together these data suggest that the effect of opioid peptide agonists and antagonists, which depend upon the level of sexual activity, may ultimately reflect basal testosterone secretion. Ebling et al. (1987) have suggested that seasonal regulation of LH release in rams relative to endogenous opioid activity depends upon a background of testosterone release. Presumably, the higher testosterone from the sexually active ram with enhanced LH release (4) would provide one explanation of the effectiveness of naloxone in the sexually active *vs.* inactive male. Additional studies in which naloxone is evaluated at higher doses may help to further elucidate the role of opioid peptides. Naloxone at the level given in this study however may be useful for identifying sexually inactive rams through effects on LH release and further studies seem warranted.

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[2,3]Reprint requests: US Sheep Experiment Station, Box 2010, HC 62, Dubois, Idaho 83423

[4]Present address: Department of Psychology, Carroll College, Helena, Montana 59625

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